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METHOD DEVELOPMENT AND VALIDATION OF A FAST AND SENSITIVE HPLC AND UV-VIS SPECTROPHOTOMETRIC TECHNIQUE FOR THE ESTIMATION OF RIVASTIGMINE IN BULK AND FORMULATION

C. SAI KUMAR[1], M. MANORAMA[2*], L. SIVA SANKAR REDDY[3], C. MadhuSudhana Chetty[4]

[1]M.Pharmacy, Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, NH-40, Nandyal-518112, Andhra Pradesh, India.
[2]Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, NH-40, Nandyal-518112, Andhra Pradesh, India.
Email: ruthmanorama5@gmail.com
[3]Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, NH-40, Nandyal-518112, Andhra Pradesh, India.
Email: lsivasankarreddy@srcpnandyal.edu.in
[4]Principal, Santhiram College of Pharmacy, NH-40, Nandyal-518112, Andhra Pradesh, India.
***Corresponding author**
M. MANORAMA
Assistant Professor, Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, NH-40, Nandyal-518112, Andhra Pradesh, India.
Email: saikumarchavadi@gmail.com

Abstract:

This study developed and validated rapid, sensitive analytical methods for Rivastigmine Tartrate, a reversible cholinesterase inhibitor used in Alzheimer's treatment. Utilizing principles of analytical chemistry to ensure pharmaceutical quality, three techniques were evaluated: HPLC, UV, and Visible Spectrophotometry.

The HPLC method employed a Zorbax XDB C18 column with a 70:30 buffer-to-methanol isocratic mobile phase, achieving a retention time of ~5.7 minutes. Spectrophotometric analysis included a UV method validated at 263.29 nm and a visible method using Bromocresol green at pH 4. All methods were validated per ICH guidelines for accuracy, precision, linearity, and robustness.

Validated per ICH guidelines, all methods met strict criteria for accuracy, precision, linearity, and robustness. The results confirm these procedures are rapid, sensitive, and reliable for the quantitative analysis of Rivastigmine in both bulk drugs and dosage forms.

Keywords: Rivastigmine Tartrate; HPLC; Method Validation; ICH Guidelines; Spectrophotometry

Introduction :

Analytical chemistry is the science of determining material composition through the chemical characterization of matter. It is categorized into:

- **Qualitative Analysis:** Identifies the chemical species present in a sample.
- **Quantitative Analysis:** Determines the relative amount of these species.

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HPLC is a versatile separation technique valued for its resolving power, speed, and ability to achieve nanomolecular detection levels. It is extensively utilized in pharmaceutical research, development, and quality control of final dosage forms.

Principles and Modes of Chromatography

Chromatographic separation relies on the differing affinities of solutes between a stationary and a mobile phase.

Types of Chromatography

- **Adsorption:** Solutes separate based on their affinity for the surface of a solid stationary phase versus a liquid or gas mobile phase.
- **Partition:** Based on the distribution of substances between two immiscible liquids.
- **Ion Exchange:** Retention depends on the attraction between solute ions and charged sites on the stationary phase.
- **Size Exclusion (SEC):** Separates particles based on molecular size using an inert gel.

Operational Modes

Chromatographic operations generally follow three techniques: **Elution** (Isocratic or Gradient methods), **Frontal**, and **Displacement**.

Analytical Method Development

Method development is required for new products without official methods or to optimize existing methods for better precision, cost, and time.

Key Steps in Development:

1. **Standard Analyte Characterization:** Collecting data on the drug's structure, solubility, and stability.
2. **Literature Search:** Surveying existing methodologies and physical properties.
3. **Optimization:** Systematically changing one parameter at a time to isolate ideal conditions.
4. **Recovery Determination:** Ensuring the method can reproducibly recover the analyte from the sample matrix.

Analytical Method Validation

Validation provides documented evidence that a specific process consistently produces results meeting pre-determined quality attributes.

ICH Validation Parameters

The International Council for Harmonisation (ICH) defines several critical parameters:

- **Accuracy:** Assessed using a minimum of nine determinations across three concentration levels.

- **Linearity:** Established using a minimum of five concentrations, typically ranging from 25% to 150% for drug assays.
- **Precision:** Evaluated at three levels: repeatability (short-term), intermediate precision (variation in days/analysts), and reproducibility (inter-laboratory).
- **Sensitivity:** Defined by the **Limit of Detection (LOD)** (lowest detectable concentration) and **Limit of Quantification (LOQ)** (lowest concentration estimable with acceptable accuracy).

System Suitability and Peak Analysis

Before sample analysis, system suitability tests ensure the equipment and procedure are capable of providing quality data.

Formulas for Suitability

- **Resolution (Rs):** Measures the separation between two adjacent peaks.

$$R_s = \frac{2(t_{R2} - t_{R1})}{W_1 + W_2}$$

- **Theoretical Plate Number (N):** A measure of column efficiency.

$$N = 16(t_R/w)^2$$

- **Asymmetry Factor (As):** Calculated as b/a at 5% or 10% peak height to measure peak tailing or fronting.

$$A_S = b/a$$

Materials and Methods:

Materials:

Rivastigmine and analytical grade reagents including sodium octane sulfonate, methanol, hydrochloric acid, ortho-phosphoric acid, and distilled/HPLC-grade water were used. All chemicals were procured from standard commercial sources.

Rivastigmine tartrate is a reversible cholinesterase inhibitor used in the treatment of mild to moderate Alzheimer's disease. Chemically, it is (S)-N-ethyl-N-methyl-3-[1-(dimethylamino)ethyl] phenyl carbamate hydrogen tartrate with a molecular weight of 400.43.

Instruments:

Analysis was performed using Shimadzu HPLC (LC-20AD), Shimadzu UV–Visible spectrophotometer (UV-1800), FTIR (Bruker Alpha), electronic balance (Shimadzu ATY224), and standard laboratory equipment.

Analytical Methods

1. UV Spectrophotometric Method (Direct Absorbance)

Rivastigmine standard solution was prepared in 0.1 N HCl and scanned in the range of 200–400 nm to determine λmax (~263 nm). Calibration curves were constructed over 10–160 µg/mL.

Validation:

Linearity, system precision, and method precision were evaluated. Assay of tablet formulation was performed using standard dilution techniques, and drug content was calculated. Accuracy was confirmed by recovery studies at different concentration levels.

2. Visible Spectrophotometric Method

A colorimetric method using bromocresol green at pH 4 with chloroform extraction was employed.

Procedure:

Standard and sample solutions were treated with buffer and dye, followed by extraction with chloroform and absorbance measurement.

Validation:

Linearity (10–60 µg/mL), precision, and recovery studies were performed. Statistical parameters such as standard deviation, standard error, and coefficient of variation were calculated.

3. HPLC Method

Chromatographic analysis was carried out using a C18 column with a mobile phase consisting of buffer (sodium octane sulfonate, pH 3) and methanol in optimized ratios (75:25 or 70:30), at a flow rate of 0.3 mL/min. Detection was performed at 210 nm with a run time of 10 min.

Procedure:

Sample injection volume was 5 µL, and retention time of Rivastigmine was ~5.7 min.

Validation:

Method validation included linearity (25–150%), system precision, method precision, and accuracy (recovery studies). Assay results were calculated based on peak area comparison between standard and sample.

Statistical Analysis

All experiments were performed in replicates, and results were expressed as mean ± standard deviation. Accuracy was evaluated by percentage recovery.

Results and Discussion

UV Spectrophotometric Method

Rivastigmine showed λmax at 263.29 nm and 269.43 nm. The method exhibited good linearity in the range of 10–160 µg/mL with a correlation coefficient of 0.998. Precision studies showed acceptable reproducibility. Assay results indicated drug content within 98.8%–103%, and recovery studies (98.4%–102%) confirmed accuracy.

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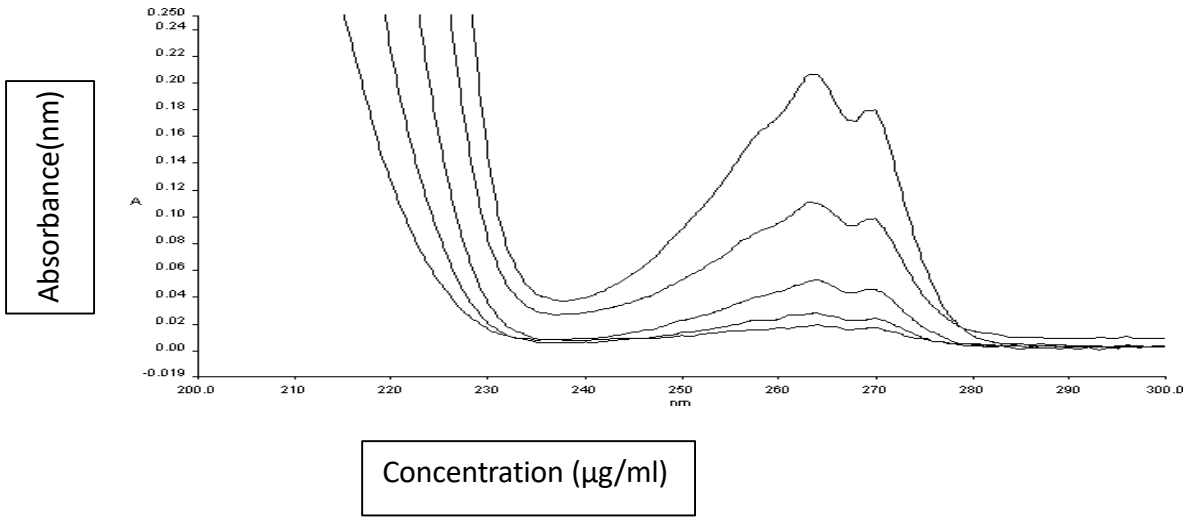

Figure: 1 Spectrum showing linearity of Rivastigmine at 263.29nm

Table: 1 Absorbance values of Rivastigmine

Sl. No	Concentration (µg/ml)	Absorbance at 263.29nm	Absorbance at 269.43nm
1	10	0.0189	0.0171
2	20	0.0280	0.0240
3	40	0.0517	0.0461
4	80	0.1112	0.0986
5	160	0.2062	0.1797

Table: 2 Correlation Coefficient, intercept and slope

Drug Name	Rivastigmine
Correlation Coefficient	0.998
Slope	900.48
Intercept	0.002

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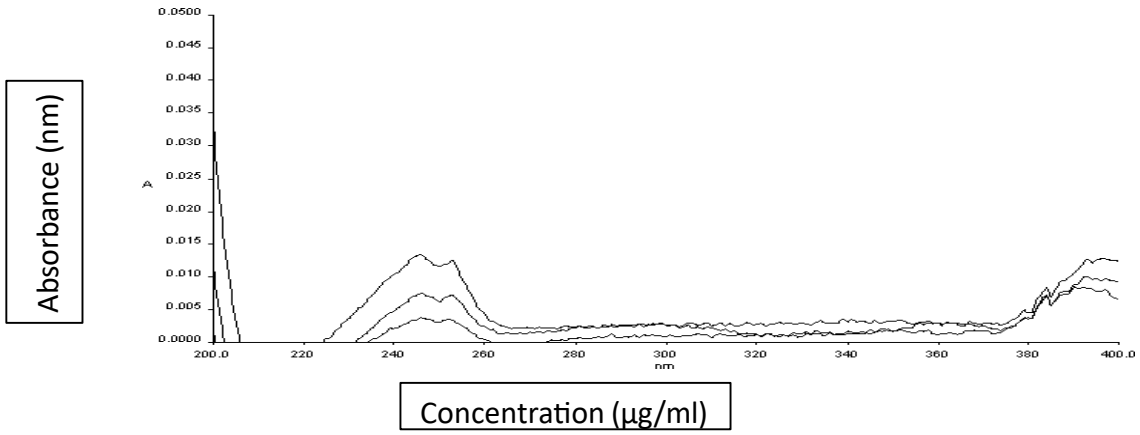

Figure: 2 Assay spectrum of Rivastigmine

Table: 3 Absorbance value Obtained for Rivastigmine

Drug	Sample no.	Amount Estimated (µg/ml)	Percentage purity
Rivastigmine	1	4.45	98.8%
	2	4.49	99.7%
	3	4.83	103%

Table: 4 Recovery studies Absorbance values of Rivastigmine

Drug	Sample no.	Amount of Std. Drug Present (µg/ml)	Amount Recovered (µg/ml)	% Recovery
Rivastigmine	1	5µg	4.6	102.04
	2	10µg	4.4	98.4
	3	15µg	4.5	100.2

Visible Spectrophotometric Method

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The λmax was observed at 415.64 nm. The method was linear over 10–60 µg/mL with a correlation coefficient of 0.999. Precision (%RSD < 1%) demonstrated reliability. Assay values ranged from 98.2% to 99.3%, and recovery results (97–103%) confirmed method accuracy.

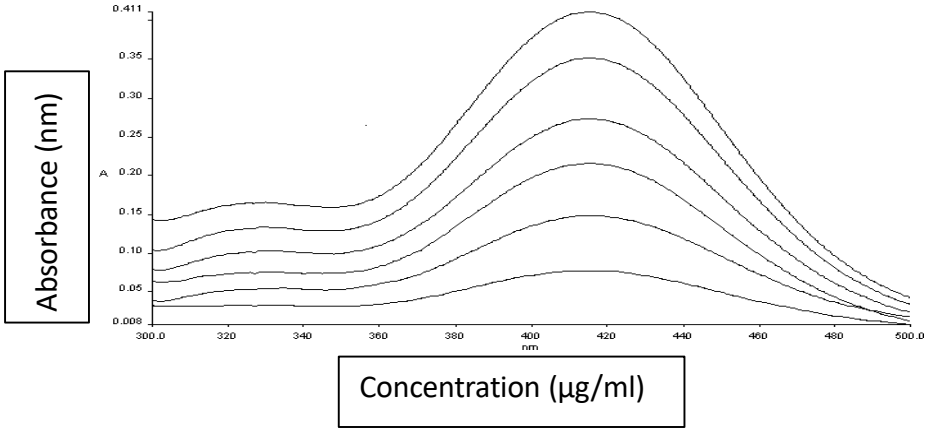

Figure: 3 Overlay spectrum of Rivastigmine for Linearity

Table: 5 Absorbance values of linearity for Rivastigmine

s. no	Concentration in µg/ml	Absorbance at 415.64nm
1	10	0.07738
2	20	0.14858
3	30	0.21905
4	40	0.27368
5	50	0.35194
6	60	0.41133

Table: 6 Correlation co-efficient, Slope, Intercept

Drug name	Rivastigmine
Correlation coefficient	0.999
Slope	14.97
Intercept	0.101

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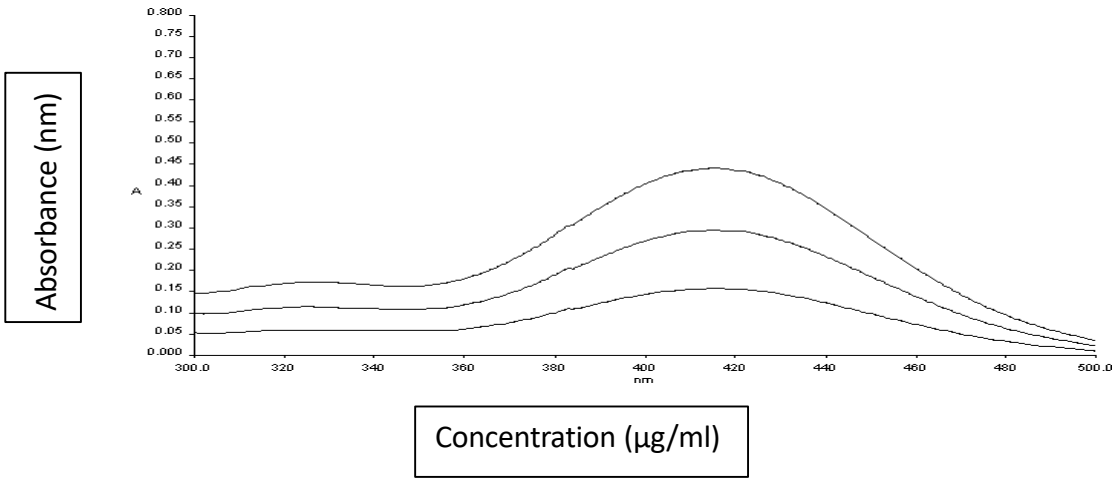

Figure: 4 Overlay Spectrum of Rivastigmine for Assay

Table: 7 Percentage purity

Drug	Sample No.	Amount estimated (µg/ml)	Percentage Purity
Rivastigmine Tartrate	1	4.47	99.3%
	2	4.43	98.4%
	3	4.42	98.2%

Table: 8 Percentage Recovery of Rivastigmine

Drug	Sample no.	Amount of sample added(µg/ml)	Amount of Std. Drug present (µg/ml)	Amount Recovered (µg/ml)	% Recovery
Rivastigmine	1	45	0	44.9	99.8
	2	45	5	44.8	99.5
	3	45	10	45.5	101.1

	4	45	15	4.60	103.0

HPLC Method

The developed HPLC method showed good system suitability (%RSD < 2%) and high linearity (r ≈ 0.999) over 25–150% concentration range. No interference from excipients was observed, confirming specificity.

Precision studies showed %RSD < 2%, indicating reproducibility. Accuracy studies demonstrated recovery within 97–103%. Ruggedness and robustness studies confirmed the method's reliability under varied conditions (flow rate, pH, temperature, and mobile phase composition).

Table: 9 System Suitability

Injection Number	Area Response
1	1439589
2	1441287
3	1441398
4	1436597
5	1439319
6	1434874
7	1439742
8	1437928
9	1434889
10	1439538
AVG	**1438516**
% RSD	**0.17**

Table: 10 showing system precision

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Concentration (%)	Area
25	365605
50	739933
75	1133123
100	1447983
125	1903406
150	2252106
Correlation Coefficient	**0.99932**

Table: 11 Specificity Placebo Interference:

Sample No	% Interference
1	Nil
2	Nil
3	Nil

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LINEARITY OF DETECTOR RESPONCE

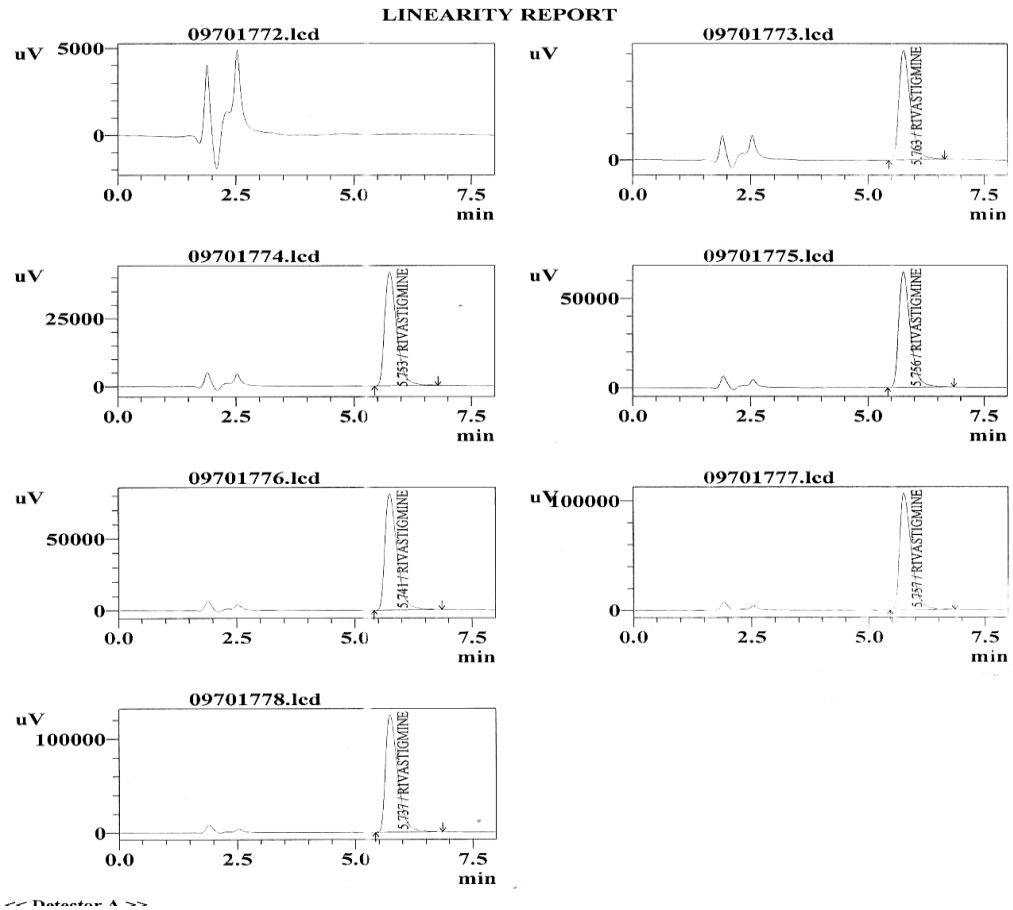

Table: 12 METHOD PRECISION

Sample No	% Assay of Rivastigmine (TRIAL-I)	% Assay of Rivastigmine (TRIAL-II)
1	101.1	99.7
2	100.5	99.7
3	100.3	99.5
4	100.6	99.6
5	100.8	99.9

6	99.6	99.4
Mean	**100.5**	**99.6**
%RSD	**0.5**	**0.2**

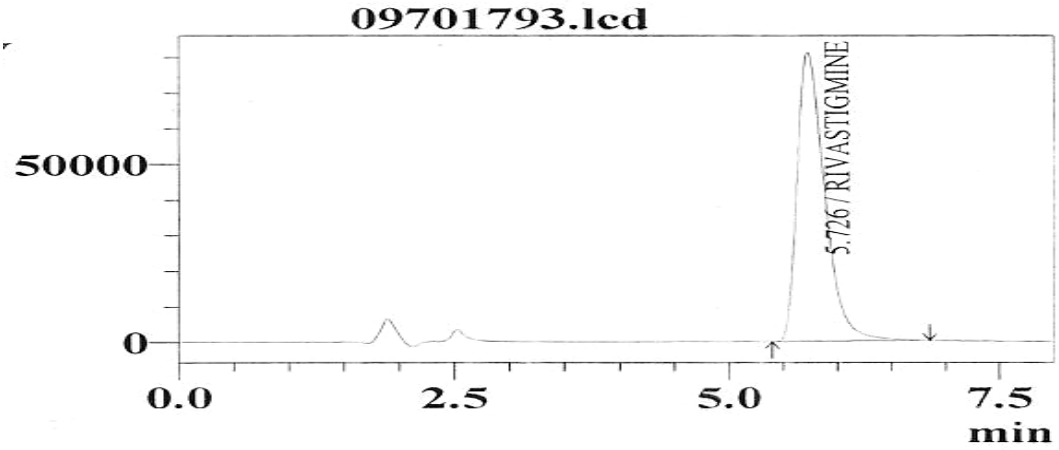

Figure: 5 Chromatogram showing Method Precision

Table: 13 values for Accuracy

Sample no.	Recovery	Wt. of Placebo Added	Wt. of Rivastigmine API added (mg)	Test Area	Recovered	% recovery
`1	50%	355.90	8.57	701200	8.45	98.7
2		355.90	8.57	702541	8.47	98.9
3		355.90	8.57	700447	8.45	98.6
1	75%	352.62	12.04	992424	11.97	99.5
2		352.62	12.04	991603	11.96	99.4
3		352.62	12.04	987990	11.91	99.0
1	100%	347.97	16.84	1376136	16.60	98.6
2		347.97	16.84	1374737	16.58	98.5

3		347.97	16.84	1368790	16.52	98.1
1	125%	343.11	20.97	1722940	20.80	99.2
2		343.11	20.97	1719947	20.76	99.0
3		343.11	20.97	1724252	20.80	99.2
1	150%	339.07	24.8	202425	24.42	98.5
2		339.07	24.8	2024712	24.42	98.5
3		339.07	24.8	2026771	24.45	98.6

Robustness:

Effect of Variation in pH of buffer

Table: 14 Effect of Variation in pH of buffer

System Suitability Parameters	Observed Value			Acceptance criteria
	PH 2.5	PH 3.0	PH 3.5	
% RSD Rivastigmine peak area of five replicate injections of standard preparation	1.20	0.06	0.19	NMT 2.0%
	0.17	0.11	0.10	

From the above study, it was established that the allowable variation in pH of buffer is from 2.8 to 3.2.

Effect of Variation in flow Rate:

Table: 15 Effect of Variation in flow Rate

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System Suitability Parameters	Observed Value			Acceptance criteria
	0.2 ml/min	0.3 ml/min	0.4 ml/min	
% RSD Rivastigmine peak area of five replicate injections of standard preparation	0.05	0.12	0.46	NMT 2.0 %
	0.09	0.07	1.33	

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Acceptance criteria:

From the above study, it was established that the allowable variation in flow rate is from 0.2 ml/min to 0.4 ml/min.

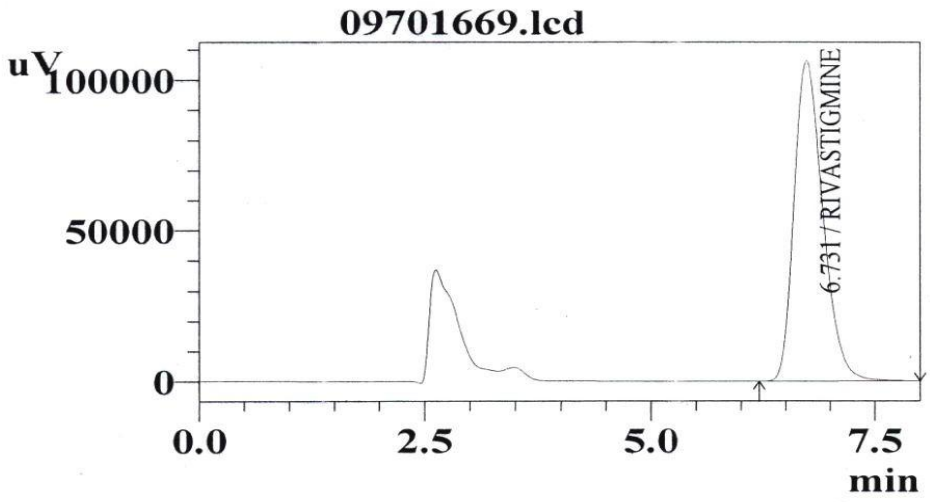

Figure: 6 Chromatogram showing Flow rate 0.2ml/min

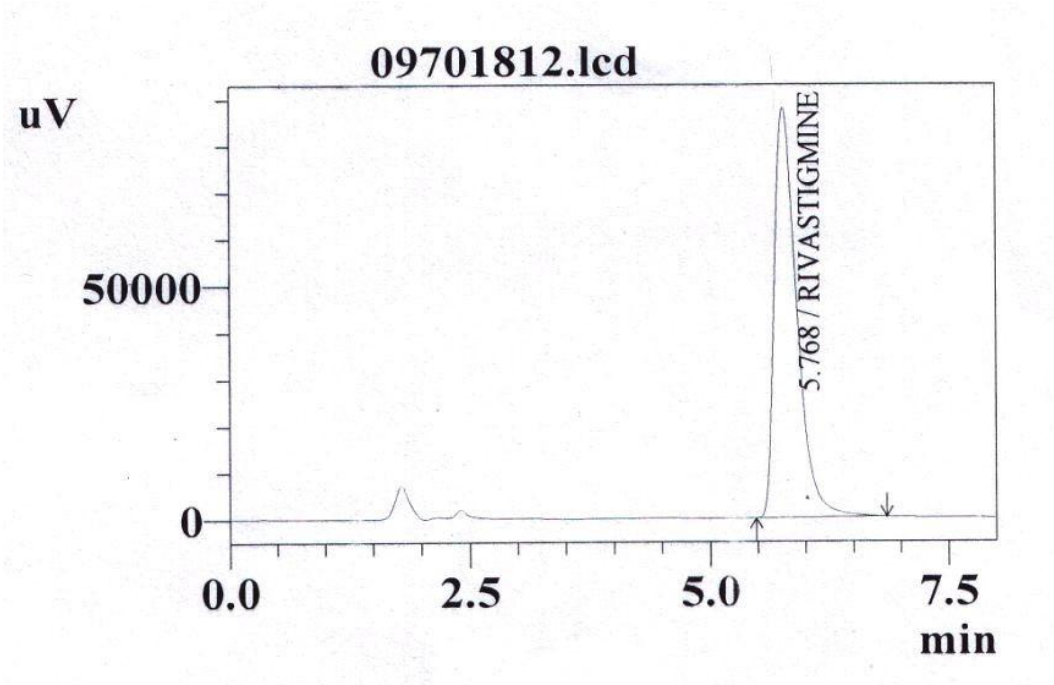

Figure: 7 Chromatogram showing Flow rate 0.3ml/min

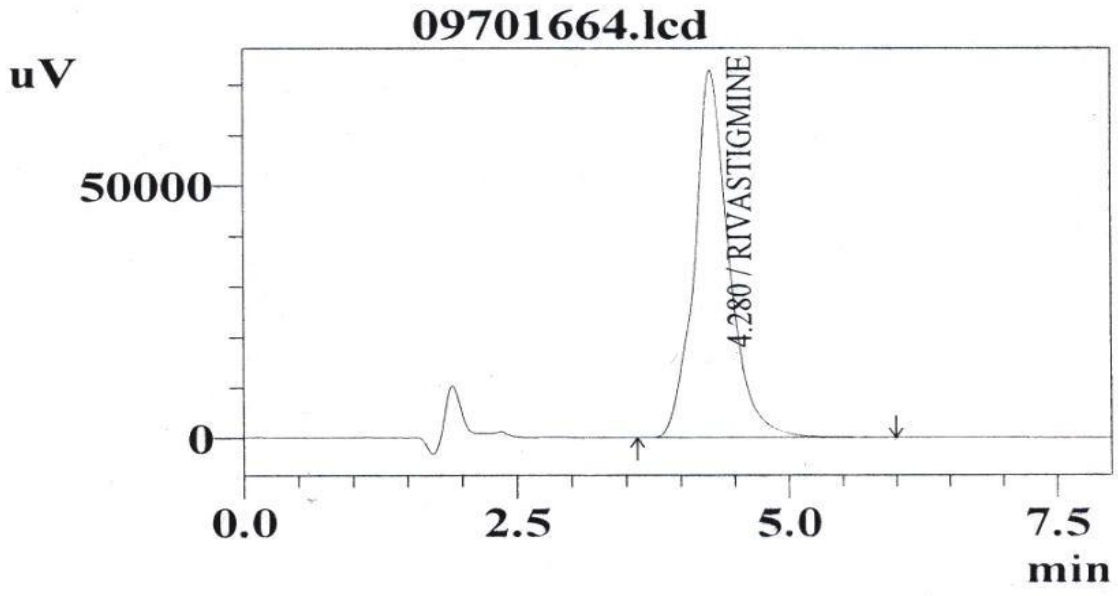

Figure: 8 Chromatogram showing Flow rate 0.4ml/min

Conclusion:

A simple, precise, accurate, and reproducible analytical approach was developed for the estimation of Rivastigmine tartrate using UV, visible spectrophotometric, and HPLC methods.

All methods showed good linearity, precision (%RSD < 2%), and accuracy (recovery within 97–103%). The HPLC method demonstrated high specificity with no interference from excipients. Robustness and ruggedness studies confirmed method reliability.

Overall, the developed methods are rapid, cost-effective, and suitable for routine quality control analysis of Rivastigmine in pharmaceutical formulations.

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